

APPENDIX A
CLAIMS SUPPORT CHART

New Claim	Support: specification page(line number)
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49	10(6-7); 10(10)-11(9); 12(28)-13(13); 13(29-31); 14(4)-16(10); 16(12-28); 18(25-26); 27(13-22); original Claim 1 and 10
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52	26(1)-27(11)
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APPENDIX B
Clean Copy of Claims Upon Entry of Preliminary Amendment

47. [NEW] A method of identifying a nucleotide sequence in a target nucleic acid, comprising the steps of:

(a) contacting a target nucleic acid with a set of immobilized oligonucleotide probe(s) and at least one labeled oligonucleotide probe from a set of labeled oligonucleotide probes under hybridization conditions effective to permit hybridization between: (i) complementary sequences of the target nucleic acid and the immobilized probes and (ii) complementary sequences of the target nucleic acid and the labeled probe(s);

(b) covalently joining immobilized probe(s) and labeled probe(s) which are adjacently hybridized to the same target nucleic acid molecule;

(c) detecting the labels of the labeled oligonucleotide probe(s) that are covalently joined to the immobilized probe(s); and

(d) identifying at least one nucleotide sequence in the target nucleic acid by steps comprising connecting the nucleotide sequences of the detected labeled oligonucleotide probe(s) with the nucleotide sequences of their respective joined immobilized oligonucleotide probe(s).

48.[NEW] A method according to claim 47 wherein the set of immobilized oligonucleotide probes comprises a first set of immobilized oligonucleotide probes and the at least one labeled oligonucleotide probe comprises a first labeled oligonucleotide probe, the method further comprising the step of, between steps a) and b): contacting the target nucleic acid with a second set of immobilized oligonucleotide probe(s) and a second labeled oligonucleotide probe under hybridization conditions effective to permit hybridization between: (i) complementary sequences of the target nucleic acid and the immobilized probes of the second set and (ii) complementary sequences of the target nucleic acid and the second labeled oligonucleotide probe, wherein the first and second labeled oligonucleotide probes comprise different nucleotide sequences and the labels of the first and second labeled oligonucleotide probes are the same.

49. [NEW] The method of claim 47 wherein said labels are detected *in situ*.

50. [NEW] The method of claim 47, wherein said covalently joining immobilized probe(s) and labeled probe(s) comprises contacting said probes with a ligating agent.

51. [NEW] The method of claim 50 wherein said labeled probe(s) are contacted with the target nucleic acid at the same time as said ligating agent.

52. [NEW] The method of claim 47, wherein after step (b) and before step (c), labeled probes that are not covalently joined to an immobilized probe are removed.

53. [NEW] The method of claim 47 in which labeled probes that are not covalently joined to an immobilized probe are removed under stringent washing conditions.

54. [NEW] The method of claim 47 in which a plurality of immobilized probes are immobilized on the same support.

55. [NEW] The method of claim 47 in which immobilized probes having different nucleotide sequences are immobilized on different supports.

56. [NEW] The method of claim 47 in which the immobilized oligonucleotide probes comprises a plurality of arrays arranged in the form of a sequencing chip.

57. [NEW] The method of claim 48 in which a plurality of immobilized probes of the first set are immobilized on a support and/or a plurality of immobilized probes of the second set are immobilized on the same support.

58. [NEW] The method of claim 57 in which the first and second sets of immobilized probes comprise a sequencing chip.

59. [NEW] The method of claim 48 in which immobilized probes of the first set having different nucleotide sequences are immobilized on different supports and/or immobilized probes of the second set having different nucleotide sequences are immobilized on different supports.

60. [NEW] The method of claim 47 in which in step (a), the target nucleic acid is contacted with a set of labeled oligonucleotide probes in a sequential manner, one labeled oligonucleotide probe at a time.

61. [NEW] The method of claim 60 in which in step (a), labeled oligonucleotide probes of the set which have different nucleotide sequences are labeled with the same label.

62. [NEW] The method of claim 60 in which in step (a), at least two labeled oligonucleotide probes of the set which have different nucleotide sequences are labeled with different labels.

63. [NEW] The method of claim 60 in which in step (a), the target nucleic acid is contacted simultaneously with the set of immobilized probes and the labeled oligonucleotide probe.

64. [NEW] The method of claim 60 in which in step (a), the target nucleic acid is contacted first with the set of immobilized probes to form immobilized probe:target complexes and thereafter with the labeled oligonucleotide probe.

65. [NEW] The method of claim 47 in which in step (a), the target nucleic acid is contacted simultaneously with at least two labeled oligonucleotide probes of a set of labeled oligonucleotide probes, wherein said at least two labeled oligonucleotide probes are labeled with different, distinguishable labels and have different nucleotide sequences that are identifiable by the properties of their respective labels.

66. [NEW] The method of claim 65 in which in step (a), the target nucleic acid is contacted simultaneously with the set of immobilized probes and said at least two labeled oligonucleotide probes.

67. [NEW] The method of claim 65 in which in step (a), the target nucleic acid is contacted first with the set of immobilized probes to form immobilized probe:target complexes and thereafter with said at least two labeled oligonucleotide probes.

68. [NEW] The method of claim 47 in which in step (a), the target nucleic acid is contacted simultaneously with at least two labeled oligonucleotide probes of a set of labeled oligonucleotide probes, wherein said at least two labeled oligonucleotide probes are labeled with different, distinguishable labels and have different nucleotide sequences that are identifiable by the properties of their respective labels and in step (d) the nucleotide sequences of the immobilized and labeled probes are determined by observing *in situ* the properties of the labels and their relative positions within an array.

69. [NEW] The method of claim 68 in which in step (a), the target nucleic acid is contacted simultaneously with the array of immobilized probes and said at least two labeled oligonucleotide probes.

70. [NEW] The method of claim 68 in which in step (a), the target nucleic acid is contacted first with the array of immobilized probes to form immobilized probe:target complexes and thereafter with said at least two labeled oligonucleotide probes.

71. [NEW] The method of claim 47, wherein a nucleotide sequence of the target nucleic acid is assembled from overlapping combined nucleotide sequences of covalently joined immobilized and labeled probes.

72. [NEW] The method of claim 47, wherein the complete nucleotide sequence of the target nucleic acid is determined.

73. [NEW] The method of claim 47, wherein the target nucleic acid is mapped.

74. [NEW] The method of claim 47 wherein the target nucleic acid is partially sequenced.

75. [NEW] The method of claim 47, wherein the immobilized oligonucleotide probes have a length F and the labeled oligonucleotide probes have a length P, where F and P are each independently between 4 and 9 nucleotides.

76. [NEW] The method of claim 47 wherein said immobilized oligonucleotide probe(s) and/or said labeled probe(s) additionally comprise a universal base or all four bases at the terminal position thereof.

77. [NEW] The method of claim 47 wherein the target nucleic acid is fragmented prior to step (a).

78. [NEW] The method of claim 77 wherein the target nucleic acid is fragmented by restriction enzyme digestion, ultrasound treatment, NaOH treatment or low pressure shearing.

79. [NEW] The method of claim 77 wherein the target nucleic acid fragments have a length T, the immobilized oligonucleotide probes have a length F and the labeled oligonucleotide probes have a length P, where T is between 10 and 100 nucleotides and F and P are each independently between 4 and 9 nucleotides.

80. [NEW] The method of claim 79 wherein T is between 10 and 40 nucleotides.

81. [NEW] The method of claim 79 wherein T is about 20 nucleotides.

82. [NEW] The method of claim 79 wherein T is about 3 times longer than F.

83. [NEW] The method of any one of claims 79 through 82 wherein F and P are each 6 nucleotides.

84. [NEW] The method of claim 47 wherein the adjacently hybridized immobilized and labeled probe(s) are covalently joined to one another by enzymatic ligation.

85. [NEW] The method of any of the preceding claims wherein the hybridization is carried out in cycles.

86. [NEW] The method of any of the preceding claims wherein the hybridization conditions are effective to permit hybridization between target nucleic acids and only those immobilized probes and labeled probes that are perfectly complementary to a portion of the target.

87. [NEW] The method of any of the preceding claims wherein the hybridization conditions are effective to permit hybridization between only those immobilized probes and labeled probes that are capable of immediately adjacently hybridizing to the same target nucleic acid molecule.

88. [NEW] The method of any of the preceding claims wherein the target nucleic acid is a cloned DNA, a chromosomal DNA or a mRNA.

89. [NEW] The method of any of the preceding claims wherein the immobilized oligonucleotide probes are immobilized by way of covalent attachment.

90. [NEW] The method of claim 89 wherein the immobilized probes are immobilized via a phosphodiester linkage.

91. [NEW] The method of claim 89 wherein the immobilized probes are immobilized via a linker.

92. [NEW] The method of any of the preceding claims wherein the immobilized probes are immobilized on glass, polystyrene or teflon.

93. [NEW] The method of any of the preceding claims wherein the label is a radioactive isotope, non-radioactive isotope or a moiety capable of emitting light.

94. [NEW] The method of claim 89 wherein the label is a fluorescent dye.

95. [NEW] The method of any of the preceding claims wherein the target nucleic acid, an immobilized probe or a labeled probe comprises a modified base or a universal base.

96. [NEW] The method of any of the preceding claims in which the immobilized probe is reusable after said hybridization.

97. [NEW] The method of claim 96 wherein the oligonucleotides of the labeled probe comprise ribonucleotides.

98. [NEW] The method of claim 97 wherein said covalently joined labeled probe comprising ribonucleotides is removed from the immobilized probe by RNAase treatment.

99. [NEW] The method of claim 96 wherein the covalently joined labeled probe comprises a uracil base.

100. [NEW] The method of claim 99 wherein said covalently joined labeled probe comprising a uracil base is removed from the immobilized probe by uracil-DNA glycosylase treatment.

101. [NEW] The method of claim 96 wherein said labeled probe comprises a chemically cleavable bond.

102. [NEW] A kit for use in identifying a nucleotide sequence in a target nucleic acid, comprising (a) a set of immobilized oligonucleotide probes, (b) a set of solutions of labeled oligonucleotide probes, in which at least one labeled oligonucleotide probes comprise a label capable of detection without recovering the label, and (c) a ligating agent.

103. [NEW] The kit of claim 102 wherein said immobilized oligonucleotide probes are arrayed on a single support.

104. [NEW] The kit of any one of Claims 102 through 103 wherein said immobilized oligonucleotide probes are immobilized on different supports.

105. [NEW] The kit of claim 102 in which the array of immobilized oligonucleotide probes comprises a plurality of arrays arranged in the form of a sequence chip.

106. [NEW] The kit of claim 105 wherein hydrophobic segments are used between arrays.

107. [NEW] The kit of any one of claims 102 through 106 in which at least two labeled oligonucleotide probes are labeled with the same label.

108. [NEW] The kit of any one of claims 102 through 106 in which at least two labeled oligonucleotide probes are labeled with different labels.

109. [NEW] The kit of any one of claims 102 through 108 in which the immobilized oligonucleotide probes have a length F and the labeled oligonucleotide probes have a length P, where F and P are each independently between 4 and 9 nucleotides.

110. [NEW] The kit of any one of claims 102 through 108 in which said immobilized oligonucleotide probes and/or said labeled oligonucleotides probe(s) further comprise a universal base or all four bases at the terminal position thereof.

111. [NEW] The kit of any one of claims 102 through 110 in which said immobilized oligonucleotide probes and said labeled oligonucleotides are each 6 nucleotides.

112. [NEW] The kit of any one of claims 102 through 110 in which said immobilized oligonucleotide probe is about one third the length of said target.

113. [NEW] The kit of any one of claims 102 through 112 in which the ligation agent is a ligating enzyme.

114. [NEW] The kit of any one of claims 102 through 113 in which the immobilized probes are immobilized *via* a phosphodiester linkage.

115. [NEW] The kit of any one of claims 102 through 113 in which the immobilized probes are immobilized *via* a linker.

116. [NEW] The kit of any one of claims 102 through 115 in which the immobilized probes are immobilized on glass, polystyrene or teflon.

117. [NEW] The kit of any one of claims 102 through 116 in which the label is a radioactive isotope, non-radioactive isotope or a moiety capable of emitting light.

118. [NEW] The kit of any one of claims 102 through 116 in which the label is a fluorescent dye.

119. [NEW] The kit of any one of claims 102 through 118 in which the target nucleic acid, an immobilized probe or a labeled probe comprises a modified base or a universal base.

120. [NEW] The kit of any one of claims 102 through 119 in which the oligonucleotides of the labeled probe comprise ribonucleotides.

121. [NEW] The kit of any one of claims 102 through 120 in which the oligonucleotides of the labeled probe comprise a uracil base.